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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 11 JULY 1953 No. 7

TREATMENT OF UREMIA WITH FORCED HIGH CALORIE-LOW PROTEIN DIET

A carefully planned and scrupulously administered high caloric diet is of value in the treatment of both acute and chronic uremia. In patients with acute uremia where a dietary regimen is of short duration, there will be little objection to the diet when it seems to enhance the chance of ultimate recovery, even if it is unpalatable.

In patients with chronic uremia, however, it should be carefully considered whether the advantages of the regimen outweigh the additional disadvantages to the patient. We believe that a useful life is possible for patients with chronic kidney insufficiency as long as they are able to produce 1200 to 1500 ml. of urine per day, no matter how lacking in concentration. Some patients who have entered the hospital with severe renal failure and who were in deep uremia when put on the diet have returned to their homes and sometimes to work for a year or more. It is unlikely that this could have been accomplished by the management of the concomitant cardiac failure and water and electrolyte disturbances alone. Comparison of their general condition before and during the dietary regimen has led many patients to the conclusion that keeping on the diet is worth the trouble and that life without a steak for dinner has still a lot to offer.

The management of water and electrolytes in uremia, although important, will not be discussed.

The cause or causes of uremia are not known, but the fact that the artificial kidney and peritoneal lavage postpone uremic death in bilaterally nephrectomized animals to as much as sixty days and the fact that the symptoms of uremia in these animals (as in patients) are greatly reduced, prove that dialyzable retention products must play an important role in the pathogenesis of uremia.

Nearly all known retention products such as urea, uric acid and creatinine are of protein origin. It would therefore be expected that a high protein diet might have a deleterious effect on the course of experimental uremia as compared with a low protein diet. This has been proved true. For example, T. Addis *et al.* (*Arch. Int. Med.* **77,** *254 (1946)*) resected three fourths of the renal tissue in rats. During the first week after the operation the death rate varied with the amount of protein consumed. There were no deaths when no protein was given, 13 per cent after 1.67 and 43 per cent after 2.38 g. of protein were administered per day. The urea content of the rats that died averaged 500 mg. per 100 g.

G. Masson, A. C. Corcoran, and I. H. Page (*J. Lab. Clin. Med.* **34,** *925 (1949)*) used bilaterally nephrectomized rats force-fed twice daily by stomach tube. Groups individually maintained on protein, carbohydrate, or fat, and a fasting group were compared in terms of survival time and azotemia. Survival periods decreased as azotemia increased. Fasting was as deleterious as protein feeding. Survival was prolonged and azotemia lessened in carbohydrate- and fat-fed animals as compared to those fed protein or placed on a fasting regimen. We are looking forward to the time that a single "shot" will make endogenous fat available to such an extent that endogenous protein is spared. Testosterone-like substances have some influence, but are not satisfactory in practice.

A satisfactory way to dispose of retention products when they cannot be excreted would be their re-utilization in protein anabolism. Ruminants have long been

known to utilize nonprotein nitrogen. Laboratory rats on a nonprotein diet, deficient only in nonessential amino acids, can use the nitrogen of ammonium salts and certain other compounds in metabolism and growth (*Nutrition Reviews* **9,** *109 (1951)*). An efficient re-utilization would make excretion unnecessary and might be extremely valuable in the treatment of conditions with impaired kidney function.

While in man there is only sparse evidence that protein in the diet unfavorably influences the progress of renal disease as such, in animals large amounts of protein have a deleterious effect on the normal (E. M. Medlar and N. R. Blatherwick, *Am. J. Path.* **13,** *881 (1937)*; L. H. Newburgh, *Arch. Int. Med.* **24,** *359 (1919)*) and the diseased kidneys. L. E. Farr and J. E. Smadel (*Proc. Soc. Exp. Biol. Med.* **36,** *472 (1937)*), for example, produced nephrotoxic nephritis (Masugi) in rats which afterwards were kept on different isocaloric diets. After eight and one-half months, 12 of the rats on a low protein diet were alive and well (one showed residual proteinuria). However, every animal on the high protein diet developed progressive nephritis, 13 of the 15 animals succumbing to renal failure within eleven months.

The demonstration of the principle of this diet need not depend on evidence with experimental animals alone. It can be demonstrated easily as follows: a person is given 200 g. of butter and 200 g. of sugar per day (2400 calories), but nothing else. The twenty-four-hour urine is now collected and the nitrogen or urea excreted is determined. Since nearly all protein catabolites are excreted with the urine, a good idea may be had of two important facts (W. J. Kolff, *Ann. N. Y. Acad. Sci.* **56,** *107 (1952)*): (1) the total amount of retention products that depend on kidney excretion under these conditions; (2) the amount of body protein broken down to form urea. It is found that: (1) After a few days the amount of urea excreted while taking this diet becomes low, 2 to 5 g. per twenty-four hours. (2) The total amount of protein from which this urea is derived must be less than 20 g. per twenty-four hours. It is concluded that a patient with chronic nephritis when on this diet will not accumulate urea in his blood when his kidneys are able to excrete 5 g. per twenty-four hours; a patient in acute anuria who retains all the urea formed will be able to live twenty-three days before the blood urea concentration will rise to 350 mg. per cent, a level well below that at which death from uremia is to be expected (J. G. G. Borst, *Lancet* **I,** *824 (1948)*; G. M. Bull, A. M. Joekes, and K. G. Lowe, *Ibid.* **II,** *229 (1949)*).

Under the conditions of the experiment protein requirement can be met by 20 g. per day. This is far below what is generally recommended, but if 20 g. of protein are added to the butter-sugar diet, the urea excretion will stay at the same extremely low level so that nitrogen balance becomes positive; more protein is evidently not needed.

This is in accord with the findings in patients on Kempner's rice diet (W. Kempner, *No. Carolina Med. J.* **6,** *61 (1945)*). The rice diet does not contain more than 20 g. of protein and when the patient consumes it in sufficient quantities urea excretion is as low as described and nitrogen balance is achieved (E. Peschel and R. L. Peschel, *J. Clin. Invest.* **29,** *455 (1950)*; V. P. Dole *et al., Ibid.* **29,** *1189 (1950)*; A. C. Corcoran, R. D. Taylor, and I. H. Page, *Circulation* **3,** *1 (1951)*).

While the patient on a high calorie-low protein diet excretes 2 to 5 g. of urea per day, the fasting human excretes 12 g. of urea per day. The difference for patients in renal failure is evident; in the first instance they may be in urea balance with a reasonably low blood urea, in the second instance they are not.

It is sometimes thought that 100 g. of

glucose a day is sufficient carbohydrate for these patients. In fact, it is only sufficient to prevent starvation ketosis as J. L. Gamble (*Harvey Lectures* **42,** *247 (1946/1947)*) and others have shown. To obtain the full protein-sparing effect 500 g. of glucose should be given. This is somewhat impractical.

The eating of protein is often forbidden to patients in uremia. The result of this negative measure is that the patient simply eats less. He then adds to his uremia the effects of starvation which, as we have seen, unfavorably influences it, and a vicious circle is formed.

J. G. G. Borst (*Nederland. tijdschrift v. Geneeskunde* **91,** *2718 (1947)*; *Lancet, loc. cit.*) in Amsterdam seems to have been among the first to force food upon patients in uremia. The forcing and the controlling of the caloric intake is the only essential difference between Borst's regimen and older low protein diets.

In a patient severely ill, whether from acute uremia or an acute exacerbation of chronic uremia, butter-sugar emulsion or butter-soup is tried first. One hundred fifty g. of sugar and 150 g. of butter with a little flour and water are made into a creamy emulsion of 600 ml. Coffee extract or other flavors may be added, and when well prepared the taste is not unpleasant. The purpose, the principle, and importance of the diet should be carefully explained to the patient, his family and the nurse.

The nurse should give 100 ml. of the chilled emulsion six times per day, and urge the patient to drink it immediately; if he vomits in the beginning he should try again to retain it. For patients with severe vomiting or nausea, Bull *et al.* (*loc. cit.*) have advocated the administration of an emulsion of oil and sugar by extremely slow, continuous intragastric drip. Others have used it to simplify nursing care.

Commercial fat emulsions like (chocolate) lipomul oral (Upjohn) and Ediol (Schenley) may be used instead of the Borst butter-soup. Polyoxyethylenesorbitan-mono-oleate or other emulsifiers are used in the commercial emulsions.

The improvement of patients with uremia who undergo the diet is often, but not always, remarkable (W. J. Kolff, *Cleveland Clinic Quart.* **18,** *145 (1951)*; *Am J. Med.* **12,** *667 (1952)*; *Ann. N. Y. Acad. Sci., loc. cit.*). With the continuation of the diet the vomiting often subsides and the patient gains confidence. Then or sometimes earlier some of the following suggestions may be used.

Borst describes the following diet as useful in chronic uremia: butter, 100 g.; sugar, 100 g.; rice, 50 g.; flour, 125 g.; fruits, 200 g.; cocoa powder, 10 g.; tea and coffee. This diet contains 2490 calories, 4 g. nitrogen; 1280 mg. (33 mEq) potassium; and 470 mg. (15 mEq) phosphorus. Furthermore, all the ingredients of the rice diet may be used. There seems to be no reason why pepper and other condiments should be prohibited. Use of a special bread containing 2.3 per cent protein, instead of the 7 per cent usual in most white bread, was especially helpful.*

In short, with french fried potatoes, vegetables, mayonnaise, pancakes with maple syrup, ice cream and desserts, life may be worth living for the patient with uremia and his diet may be much better than what some of us had to eat during the war years in Europe.

Often it suffices to change the patient's eating habits just a little and to supplement with high calorie emulsions. More protein should be allowed if kidney function improves, or an anabolic phase supervenes. Thus more protein is prescribed if blood urea falls below 50 mg. per cent, or if a slight

*This bread, Noury bread, is made by Nourypharma, Deventer, Holland (Mr. G. Horbach). It is unfortunately not available in the United States.

increase in protein intake is not followed by an increase in blood urea.

Potassium deficiency: The diet contains little potassium and the large amounts of carbohydrate promote a shift of the extracellular potassium into the cells. This may be useful in cases of impending potassium intoxication in anuric anuria; it may become dangerous, however, in chronic uremias where additional potassium may be lost in the urine (W. J. Kolff, *J. Lab. Clin. Med.* **36,** *719 (1950)*).

Vitamin deficiency: The sad experiences in some Japanese concentration camps may be recalled where "camp-eyes" developed when the inmates secured a supply of highly purified coconut fat. Symptoms similar to acute vitamin deficiencies sometimes develop in a matter of days in severe uremia.

Too much restriction of protein: This may occur when an overzealous housewife takes the "must-nots" of the diet more seriously than the musts.

Severe infections, intoxication (bichloride of mercury), crushing injuries, major operations, etc., can obscure the effect of the diet because they cause protein destruction, both directly and indirectly. It is safe to assume that the principles of the diet must prevail as soon as tissue destruction begins to subside.

Sometimes the resistance of the patient to some ingredients of the diet is such that they should be omitted and replaced by others. Conferences among patient, dietitian and physician are necessary to find the optimum diet for every patient. Sometimes a 2000 calorie diet cannot be fed. We then try to give as much carbohydrate and fat as is possible.

In conclusion, the patient in uremia may advantageously be treated with a high calorie-low protein diet; no more than 20 g. of protein a day is needed when caloric requirements are fulfilled with carbohydrate and fat. The diet must be controlled and calculated as are diabetic diets, the difference being that while the diabetic patient wants to eat too much, the uremic patient wants to eat too little. He should therefore be urged to eat.

The effectiveness of the method with which the diet is being administered may be controlled by daily determination of urinary urea excretion. In absence of causes for protein destruction, it should not exceed an average of 5 g. per twenty-four hours.

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EFFECT ON APPETITE OF DIETARY TREATMENT OF HYPERTENSION

So often in research results of a study designed to prove or disprove a hypothesis will turn out to be of much greater interest in some apparently unrelated field. This seems true of the investigations of V. P. Dole and co-workers (*J. Clin. Invest.* **32,** *185 (1953)*) in their third report concerned with the dietary treatment of hypertension. The first article in the series (*Nutrition Reviews* **9,** *73 (1951)*) was concerned with hypertensive patients maintained on the Kempner rice-fruit diet. It was observed that weight loss and a negative nitrogen balance occurred during an initial period, but that most patients then stabilized their weight and nitrogen balance, and came into equilibrium. This adaptation usually required months. In the second study (*Ibid.* **10,** *5 (1952)*) weight loss was observed, but it did not become constant, except perhaps during the higher protein feedings. The authors considered that the weight loss was most probably due to limitation of protein in the diet. In no instance were wide variations in sodium intake effective in changing weight.

In the most recent study the conjecture that the level of dietary protein intake was in some way related to changes of body weight of 6 patients with uncomplicated hypertension was examined. The basic diet, not described in detail, was severely restricted in sodium (4 mEq per day). This diet provided about 35 g. of protein per day. Variations in protein content of the diet were made, apparently by changing the intake of a low-sodium modified milk supplement. Large increases in the sodium intake per day were made by giving salt in gelatin capsules. An unlimited supply of carbohydrate and fat was offered to each patient.

The studies were designed with an initial control period when the basal diet was fortified by the addition of the protein supplement and sodium chloride. During the second period this protein level was maintained while the sodium supplement was eliminated. In the third period the protein intake was restricted to about 35 g. per day in addition to the sodium restriction. In 2 patients further restriction of dietary protein to 8 g. per day was instituted. Finally, the protein in the diet was increased in all but 2 patients.

Changes in diet often produced falls in blood pressure which were clearly related to the removal of sodium from the diet and not to the protein content. This conclusion had previously been reached by these authors and by others.

Somewhat unexpected were the changes of appetite and weight which seemed to be related to changes in the quantity of protein provided per day. These 6 patients were allowed to eat calories as their appetites dictated, but the protein content of their diet was kept constant in each period. It was observed that these individuals altered their caloric intake in direct relation to changes in the level of dietary protein. The magnitude of this spontaneous change in calories amounted to 14.6 calories per gram change in protein intake, which, as the authors point out, is considerably greater than the caloric value of the protein alone (4.1 calories per gram). Since the caloric intake depended upon the individual's desire to eat, it appeared that protein in some way influenced appetite. The authors point out that it seems paradoxic that the patients should lose weight when changed from one adequate diet to another, and suggest two possible explanations. The first is that time is required for the organism to increase its efficiency, and during this period of adaptation tissue is lost because of wasteful expenditure. The alternative given is that loss of tissue may be essential to the adaptation, being in fact a part of metabolic adjustment. The authors believe this latter explanation is the more logical. The hypothesis proposed is as follows: The body maintains a steady state of composition that consists of many substances in ceaseless flux, forming a set of interacting rate processes. A change in the rate of one process would change the inventory of metabolites that share in other rate processes. Following such a change a new steady state might or might not be reached, depending upon whether the changes in inventory bring the rates into balance. Thus relatively small changes in intake of one nutrient might call for a considerable modification of the composition of the body.

There seems to be little doubt that the ad libitum caloric intake of these patients was directly proportional to the regulated protein intake, although of course there was some overlapping. Whether the change was due entirely to the quantitative change in protein per day, or whether a more subtle qualitative change in protein or associated nutrients could account for it would require careful analysis of the diet as to its adequacy in all nutrients, especially vitamins and essential amino acids. Unfortunately, the precise composition of the diets is not presented by the authors. This is particularly significant since restriction of the sodium content of the diet to 4 mEq per

day may limit the supply of other nutrients (*J. Am. Med. Assn.* **149,** *1317 (1952)*). Also pertinent in this regard is the insufficient knowledge of the vitamin, mineral and amino acid content of the modified milk used as the protein supplement. The composition of the modified milk powder supplied is not presented other than with respect to its protein, carbohydrate, fat and sodium content. Thus, it may be that the diet was low or restricted in nutrients other than protein, and that this may have had an effect upon the appetite. If this is true, it is of considerable interest that this led to partial anorexia in short periods of time; and, as the authors point out, such alterations in the diet may have some value in the treatment of obesity. Of course, diets inadequate in essential nutrients will eventually cause a loss of weight, but cannot be considered as satisfactory for the treatment of obesity. The authors believe that the diets used here must be considered adequate, but the published evidence is not convincing, since the actual food consumption is not given.

It seems clear that under the conditions of this study alterations in the protein intake produced changes in appetite and hence in caloric intake and finally in body weight.

SENILE OSTEOPOROSIS AND CALCIUM INTAKE

Among the changes which are frequently seen in man as age increases is the rarefaction of bone known as osteoporosis. Although the mechanism by which this occurs is not fully understood, the most commonly accepted hypothesis is that of Albright and his colleagues (F. Albright and E. C. Reifenstein, "The Parathyroid Glands and Metabolic Bone Disease," *Williams and Wilkins Co., Baltimore (1948)*). It is proposed that osteoporosis is characterized by a defective synthesis of protein matrix which prevents replacement of bone lost in the course of usual metabolic turnover.

The role of calcium deficiency in this phenomenon has been poorly understood, although its importance has been suggested by several workers (*Nutrition Reviews* **10,** *97 (1952)*). The reports available concerned with the actual calcium intakes of elderly persons (*Nutrition Reviews* **9,** *304 (1951)*) reveal figures which are under the recommended allowances in a high percentage of such individuals, although it should be clearly understood that such data have been largely obtained from institutionalized groups. More recent studies in Denmark by N. Vinther-Paulsen (*Geriatrics* **8,** *76 (1953)*) have been undertaken in a group of such institutionalized elderly patients to determine the relationship, if any, between calcium intake and osteoporosis.

Thirty-eight patients, 5 of whom were males, aged 68 to 96 years, were followed during two seven-day periods in which calcium and phosphorus dietary intakes were calculated. Most of the subjects were suffering from heart disease, senile dementia, central nervous system syphilis, or cerebral vascular accidents. In addition to dietary calculations, radiologic studies of the vertebrae, and blood calcium, phosphorus and alkaline phosphatase determinations were carried out.

The ingestion of calcium per day varied between 0.2 and 1.1 g. and of phosphorus from 0.45 to 1.7 g. Of 19 subjects with calcium intakes of 0.5 g. or less, 74 per cent revealed radiologic evidence of decalcification, while among those who ingested over 0.5 g. the incidence was but 14 per cent. Approximately similar findings were recorded in regard to phosphorus intake, with 64 per cent showing decalcification in the group ingesting 0.9 g. or less, whereas only 9 per cent showed this bone change in the group taking more than this amount. The

blood determinations showed no relationship to the bone lesions and were for the most part within normal limits. Two patients had elevated alkaline phosphatase values with evidence of decalcification, but the authors could give no explanation of these findings.

Although the authors state that care was taken to select patients who were eating with the same appetite that they had before the experimental period, and that the diet contained milk and cheese ad libitum, caution should be exercised in the interpretation of these results. The subjects under study were ill patients subject to all the feeding handicaps which are found in such persons. As a result their actual intakes may have been reduced in comparison to those of previous years by the illness which they suffered. Perhaps more important is the lack of data in regard to protein intake, particularly since the role of this factor has been emphasized by Albright (*loc. cit.*). It would appear that failure to eat calcium and phosphorus as it is contained in milk and cheese may also have led to deficient protein intakes. Thus, the correlation of decalcification of bone with low calcium and phosphorus intake may be no greater than the correlation of this finding with low intakes of other nutrients, particularly protein.

The high frequency of poor calcium intake in relation to osteoporosis among elderly institutionalized individuals deserves further investigation. If a causal relationship can be demonstrated, there is hope that at least part of the disease resulting from bone demineralization can be prevented.

WHEAT GLUTEN AND THE CELIAC SYNDROME

For the morphologist who feels uncomfortable whenever he is unable to relate a disease to some specific pathologic lesion, the celiac syndrome has always been a particularly frustrating entity. Indeed, the entire group of diseases of intestinal absorption which T. E. Hess Thaysen termed "idiopathic steatorrheas" (*Quart. J. Med.* **4,** *359* (*1935*)), and of which the celiac syndrome is one, has been extremely disappointing from the morphologic standpoint: no consistent histologic findings can be brought forth to explain the defect in intestinal absorption which so frequently has caused inanition and even death in patients.

The celiac syndrome ordinarily manifests itself between the first and fifth years of life. Its onset is usually gradual and when the illness is fully developed it is characterized by marked emaciation of the trunk and extremities, distended abdomen, seborrheic dermatitis of the scalp, and alteration of mental state. The feces are voluminous and fatty, and the course of the disease is punctuated by remissions and exacerbations.

A variety of diets has been used in treatment of the celiac syndrome. Because of the steatorrhea, fat-free diets were among the first tried and subsequently discarded. Among others, W. Sheldon (*Arch. Dis. Childhood* **24,** *81* (*1949*)) has reported that omission of starch had a favorable clinical effect and lowered the fat content of the feces. The banana diet of S. V. Haas (*Am. J. Dis. Child.* **28,** *421* (*1924*)) and the vegetable-fruit diet of G. Fanconi (*Schweiz. med. Wchnschr.* **58,** *789* (*1928*)) also enjoyed some success in the treatment of celiac patients. Despite these suggestions, progress in understanding and effectively treating the celiac syndrome has been disappointingly slow.

Several years ago, W. K. Dicke ("Coeliake," *M. D. thesis, Utrecht* (*1950*)) observed that patients with the celiac syndrome appeared to react particularly unfavorably to the presence of wheat (and rye) in the diet. As a result of this observation, J. H. van de Kamer and H. A. Weijers studied the role of wheat in the diet of celiac patients (*Voeding* **14,** *37* (*1953*)). Their

findings were dramatic. They observed that whenever the diet of such patients contained wheat, even in very small quantities, the patients became pale, the appetite disappeared, weight was lost, diarrhea occurred and steatorrhea increased. As soon as the wheat flour was left out of the diet all the unfavorable symptoms became less marked or disappeared entirely and the general condition of the child improved. Such improvement occurred even if wheat *starch* remained in the diet. Similar results were observed in 30 consecutive cases and, more recently, these findings have been confirmed by C. M. Anderson and her associates (*Lancet* **I,** *836 (1952)*), and by W. Sheldon and D. Lawson (*Ibid.* **II,** *902 (1952)*).

H. A. Weijers and J. H. van de Kamer (*Centraal Inst. v. voedingsonderzoek T.N.O. Publication No. 113, Utrecht (1950)*) demonstrated a close correlation between the harmful clinical effect of wheat flour and the amount of fat excreted daily in the feces. Their preliminary experiments indicated that the source of the trouble lay in the protein fraction of wheat, the wheat gluten, rather than in its starch portion as had been indicated in the earlier studies of D. H. Andersen (*J. Pediat.* **30,** *564 (1947)*), Sheldon (*loc. cit.*), and others. Of this fraction, the gliadin seemed to be especially implicated. Accordingly, the Dutch investigators believe that the beneficial effects of the banana diet and the fruit-vegetable diet may be explained by the dearth of wheat in these diets. Moreover, the relapses which occur while such diets are in force are also explicable because wheat and wheat products are not rigorously excluded in these regimens.

The Dutch workers went on to analyze the feces of children with celiac disease for their content of saturated and unsaturated fatty acids. In about 20 patients with celiac disease it was shown that the absorption of unsaturated fatty acids was always notably better than that of saturated fatty acids. In some instances, especially when the diet contained wheat, this difference was very striking. When oils with a relatively high content of unsaturated fatty acids such as olive oil and sunflower oil were given to celiac patients, fat absorption in the subjects under study became normal. From these findings, it seems clear that for the diet of celiac patients olive oil, soybean oil and other unsaturated oils are to be preferred to butter, beef fat and coconut oil. The favorable effect of olive oil on fat absorption in celiac children has been known for some time and is described in Holt's "Diseases of Infancy and Childhood" (L. E. Holt and R. W. McIntosh, *p. 252, D. Appleton-Century, New York (1939)*).

The next step was to determine whether the harmful influence of wheat gluten in such patients could be nullified by the concurrent incorporation into the diet of an unsaturated oil as the only source of fat. It was found, regardless of the source of the dietary fat, that wheat protein always exerted an unfavorable effect on the course of the celiac syndrome.

Weijers and his associates (*loc. cit.*) found that in certain patients with celiac disease more saturated fatty acids were being excreted than were being consumed in the diet. To these workers, the most plausible explanation of this phenomenon was that fat was being excreted into the lumen from the intestinal wall. To test this hypothesis, the Dutch scientists studied the excretion of fat in the feces of patients on diets entirely free of fat. It was learned that such patients continued to excrete fat in the feces; accordingly, the Dutch group concluded that in patients with the celiac syndrome wheat (or rye) probably precipitated a disturbance in fat metabolism, thereby bringing into clinical evidence a disease already latent and presumably congenital in origin.

The interpretation of the Dutch workers differs from that of Anderson and her associates (*loc. cit.*). According to her published view, the direct cause of the impaired absorption in diseases of the sprue-celiac syn-

drome is an excess of mucus formation in the small intestine, which prevents normal fat absorption. Moreover, Anderson explains the fecal fat which is found on a fat-free diet as being due to synthesis of fat in the intestinal tract by bacteria. Weijers and coworkers (*loc. cit.*) tested Anderson's postulate of bacterial production of fat by studying a celiac patient on a wheat-containing, fat-free diet. The patient was given oral streptomycin for a week, and the authors contend that the bacterial flora in the intestines was virtually wiped out by this treatment. No indication of a decreased fat excretion during or after streptomycin therapy was observed. Lastly, the Dutch workers followed a celiac patient on a fat-free diet during a febrile illness. They observed that fat excretion increased while the fever was present and diminished immediately after decline of the fever. It is their belief that the finding of this kind of relationship between a systemic derangement and increased steatorrhea tends to support the view that celiac disease involves a disorder of fat metabolism and not primarily mechanical impairment of fat absorption.

In view of the apparent similarity between the celiac syndrome, idiopathic steatorrhea and tropical sprue, it would seem desirable to test the important theory of the mechanism of the celiac syndrome developed by Weijers *et al.* in the other two poorly understood diseases of small bowel absorption. Whether the defect will prove to be at the level of intestinal absorption, as Anderson seems to believe, or at the metabolic level, as the Dutch workers so persuasively have argued, there can be no doubt that an extremely useful advance in the understanding and treatment of the celiac syndrome has been made.

EFFECTS OF GLUTAMIC ACID ON INTELLIGENCE

A fairly comprehensive review of the literature on the relationship between glutamic acid and mental functions was published in *Nutrition Reviews* **9,** *113 (1951)*. Probably it is the fact that predominantly negative results were obtained in the best controlled investigations which accounts for the slackening of research activities concerned with glutamic acid as a peculiarly potent "brain food." Nevertheless, several studies have been published since the above-mentioned review was written, which merit consideration.

Attention should be drawn to two extensive reviews on glutamic acid and cerebral function (H. Weil-Malherbe, *Physiol. Rev.* **30,** *549 (1950)*; H. Waelsch, *Advances in Protein Chemistry* **6,** *299 (1951)*), both focussed on the role of this amino acid in the metabolism of nervous tissue, although the psychologic effects are also considered. Weil-Malherbe (*see also Biochem. J.* **50,** *xxiii (1952)*), in view of the ready availability and presumably high rate of synthesis of glutamic acid in the brain cells, questions the validity of the view that the clinical effects are due to its action as a "brain food." He is of the opinion that these effects are probably not due to a special function in brain metabolism, but to an unspecific adrenergic action. He reported a confirmation of the finding of an increase of sympathomimetic amines in blood after the intravenous injection of glutamic acid during hypoglycemic coma. The striking similarity between the reported effects of the acid and amphetamine is brought out in a table based on the analysis of the literature. The effects on mood, attention, tempo, initiative and social attitudes and behavior are considered.

Waelsch (*loc. cit.*) considers that the mechanism of psychologic improvement observed when glutamic acid was fed is obscure. He is unwilling to accept Weil-Malherbe's thesis implying the involvement of the autonomic nervous system. To Waelsch, the

psychologic mechanism of the intellectual gain may represent an acquisition of new intellectual tools, that is, real increase in intelligence, or a better utilization of existing intellectual abilities due to improved emotional regulation. He tends to favor the second alternative.

While the interpretation remains open to debate, what progress has been made in the assemblage of facts? Waelsch (*loc. cit., p. 328*) refers to a study by K. Albert, P. Hoch, and Waelsch (*J. Nerv. Ment. Dis.* **114,** *471 (1951)*) on 50 mentally defective children, which confirmed the positive results of an early study limited to 8 mentally retarded patients ranging in chronologic age from 6 to 26 years, in mental age from 2 to 8 years, who had no history of convulsive disorder. This was to exclude the possibility that the removal of an epileptoid mechanism was the reason for the apparent improvement in mental status. The changes (improvements) observed in the second study carried out on a larger group of mental defectives were statistically significant and were considered to be due to the administration of glutamic acid (9 to 12 g. daily in three doses). As before, periods of glutamic acid and placebo administration were alternated, each period lasting four months. The study was limited to children (6 to 17 years) with very low mental ability (I.Q.'s from 18 to 58). No actual data are given, but the statement is made that "although there is no doubt that the effect of administering glutamic acid was real, the positive change in I.Q. was rarely large enough to be of practical significance in this group of defectives of low intelligence." Information is also given on a study of a smaller group of children of less subnormal intelligence (I.Q. 70 and above). In this case the daily administration of about 10 g. of glutamic acid was without effect in 8 out of 9 subjects.

The effects of glutamic acid on intelligence were also investigated by J. R. Milliken and J. L. Standen (*J. Neurol. Neurosurg. Psychiat.* **14,** *47 (1951)*). The study included two groups of mentally defective adults, one group of mentally defective children and two groups of boys of normal intelligence. Only the defective groups will be considered here. Each group was divided into a control section and an experimental section receiving a daily dose of 12 g. or more of glutamic acid, administered by mouth in water two to three times a day, with or before a meal. The control subjects received corresponding amounts of the placebo solution. The test battery contained verbal and performance tests of intelligence as well as tests of personality and a measurement of body sway. The period of administration of each solution was about fifteen weeks. There was no evidence in favor of the hypothesis that glutamic acid improves cognitive functioning, or the scores on the personality tests. There was some gain, in favor of the supplemented groups, in the body sway test, but the difference reached the 1 per cent level of statistical significance in only one out of six comparisons.

In her study of the potential value of glutamic acid in the treatment of mental deficiency, M. Foale (*J. Ment. Sci.* **98,** *483 (1952)*) compared the abstract and practical intelligence in two groups of boys matched for age (12 years 2 months in the experimental group, 12 years 6 months in the control group), average residence in the institution (ten and eleven months), average intelligence quotient (62 and 64) and average practical ability (mean scores of 76 in both groups). There were 15 boys in each group. They received the same training, care, and diet, except for the daily dose of 9 g. of glutamic acid during the first four months and 12 g. during the subsequent six months, administered to the experimental group in the form of tablets. Apparently no placebos were used with the control group.

The initial and final mean I.Q. values, obtained by equivalent forms of Cattell's test of intelligence, were 62.1 and 65.3 in the group on glutamic acid treatment and 64.1 and 64.2 in the control group. The difference

in gain (3.1 points) is of questionable significance. Statistically, it reached the 5 per cent level of significance ($F = 8.03$, for 1 and 28 degrees of freedom). It can be said that at least *some* individuals in the supplemented group showed fairly large gains (up to 11 points), while in the control groups the differences between the two testing periods ranged from a loss of 2 points to a gain of 3 points.

In Foale's study the test of "practical ability" yielded the mean score values of 76.2 and 82.1 for the experimental subjects and 76.5 and 81.7 for the controls, indicating almost identical gains (5.9 and 5.2) in both groups, interpreted as the effects of practice. The means and tests of significance were calculated by the reviewer from the author's raw data.

Mental deficiency is as much a social as a psychometric concept, overt behavior being of paramount importance as regards fitness for normal life. In this investigation the social aspects have been examined by noting general behavior in the institution and the achievement in simple domestic routine duties, at school and at work in the occupation center. In half of the boys in the experimental group a definite improvement in general adjustment was thought to be present by the author, the teachers and male nurses. Reports from these three sources are not treated separately and the validity of the observations was not cross-checked. The characterizations of the behavior changes are for the most general (more alert and self-confident, can hold his own better, more interested in work) and the time course has not been traced. Behavioral data on the control group are not presented, except for a comment that "none of the control boys showed such marked changes." Thus, the results could be interpreted as mildly positive and encouraging. However, methodologically, the study has its weaknesses. The principal question mark is the apparent absence of placebos. In an institutional setting, a "preferential" treatment consisting in the administration of the glutamic acid supplements could markedly affect the validity of the study, especially as far as behavioral responses are concerned. It is almost unavoidable that patients gain some status and receive more attention by their participation in a special project than is the average for the institution.

In view of what has just been said, the evaluation of glutamic acid therapy in mental deficiency by R. N. Zabarenko and G. S. Chambers (*Am. J. Psychiat.* **108,** *881 (1952)*) is particularly interesting. The study was meticulously planned, with an attempt to control and evaluate all other factors except the experimental variable (glutamic acid dosage). The placebos corresponded exactly in appearance to the glutamic acid tablets and were similar in taste. Both younger (ages 5 to 11) and older patients were used. When about one half of the total group was transferred from a state school in rural Pennsylvania to Pittsburgh for purposes of closer observation and more elaborate study, six months were allowed for adaptation to the new environment before the experimental period proper. During the first half of the medication period extending over at least one hundred days one half of the patients received glutamic acid, the other half placebos. In the second medication period the groups were reversed. Unavoidably, this experimental design (rural and urban environment; younger and older subjects; order of the placebo and glutamic acid administration) resulted in small subgroups.

The authors conclude categorically that the glutamic acid supplementation, increased from low (but unspecified) levels up to a daily intake of 40 g. over a period of ten days, did not produce significant gains in mental capacity, while environmental stimulation appeared to enhance mental functioning. Out of the large psychometric material (which included also tests of personality, nonverbal intelligence, and manipulative dexterity) only the mental

age scores, obtained by the Stanford-Binet and the Merrill-Palmer tests, were reported in this paper. Strangely enough, the mean values of the separate groups while on the experimental and placebo regimen were not reported and no details of the analysis of variance concerned with the crucial question of evaluating the effect of the experimental variable are given. A table containing the mental ages gained during the study by 8 boys who were placed in a particularly favorable social environment is given. The mean gains were 6.4 months on glutamic acid therapy, 4.8 months on placebos, with no statistically significant difference.

Only a general, over-all summary of the psychiatric observations was given, indicating again no gains properly ascribable to the drug therapy. Special attention has been paid in the study of behavior to the patient's alertness, irritability, insomnia and psychomotor output. No striking changes in these features were evident. Among those that have occurred there was no consistent difference in the incidence in the glutamic acid- or the placebo-treated patients. It is hoped that the results of this important study will be reported elsewhere in greater detail.

THIAMINE AND WERNICKE'S SYNDROME

The clinical manifestations of Wernicke's syndrome in man consist of paresis of extraocular muscles, nystagmus, ataxia and varying degrees of mental confusion. Certain characteristic pathologic findings are usually seen in cases coming to autopsy. In this country, the syndrome is usually associated with chronic alcoholism, but it is thought to be due to deficiency of one or more of the B-vitamins, the one most commonly implicated being thiamine. The apparent reversal of some of the clinical manifestations by the administration of thiamine and also the occurrence in animals of a similar condition known as Chastek's paralysis, which is caused by the ingestion of a thiaminase contained in raw fish (*Nutrition Reviews* **1,** *61 (1942)*), led to this conclusion.

Some doubt has been cast on the exact relation of thiamine deficiency to the pathogenesis of Wernicke's syndrome (*Nutrition Reviews* **1,** *24 (1942)*; **3,** *184 (1945)*; **8,** *50 (1950)*). These doubts are due in part to the difficulty of studying nutritional deficiencies in man by providing but one nutrient at a time. Most patients with nutritional deficiencies coming to the hospital are given not only one or more specific nutrients, but also food. Thus the studies cannot be satisfactorily controlled.

G. B. Phillips, M. Victor, R. D. Adams, and C. S. Davidson (*J. Clin. Invest.* **31,** *859 (1952)*) attempted to overrule this criticism by providing a highly purified diet for patients with Wernicke's syndrome immediately upon their admission to the hospital, thus preventing access to other nutrients. Nine patients with typical Wernicke's syndrome including ophthalmoplegia were given no food from the time of admission to the hospital for periods of three to eleven days, except for the oral administration of glucose (usually with sodium chloride and sometimes with potassium chloride). At varying times during this regimen thiamine alone was added to the purified diet. At other times, various vitamins of the B-complex, excluding thiamine, were given first. Subsequently, the regular hospital diet was provided.

Rapid improvement in the extraocular palsies occurred within one and one-quarter to six hours after thiamine was given, although sometimes several days were required for residual weakness to disappear. This response left little doubt in the authors'

minds as to the specificity of thiamine for the ophthalmoplegia. Improvement in the nystagmus after thiamine administration was more gradual, and seemed to respond to thiamine alone, although it sometimes persisted for relatively long periods of time. The ataxia likewise improved slowly, but also appeared related to a deficiency of thiamine. The authors found the mental changes the most difficult to evaluate. One patient improved somewhat after receiving multiple vitamins and thiamine, and another improved with thiamine alone. In 6 of the patients little change could be detected. However, the authors note that, since the mental disturbances improved so slowly, the failure of thiamine to alter them does not exclude thiamine deficiency as a cause.

The authors conclude that the ophthalmoplegia of Wernicke's syndrome is related to a specific lack of thiamine, but that the other phenomena are less conclusively related to the deficiency of this vitamin. These observations using the purified diet and addition of one nutrient alone, although difficult to do, clearly establish the relation of an added nutrient to those phenomena which improve rapidly enough so that observations can be made.

STUDIES ON BODY COMPOSITION

Valuable information has been accumulating on the chemical composition of the human body. In studies of 2 adult bodies, E. M. Widdowson, R. A. McCance, and C. M. Spray (*Clin. Sci.* **10,** *113 (1951)*) found that the fat content of the whole bodies, on a fresh-weight basis, was 14.9 and 23.6 per cent, respectively. This difference is especially interesting as the former was that of a man slightly overweight and the latter that of a woman who was thin. The water content of the whole bodies on a fat-free basis was 72.5 and 73.2 per cent, respectively. Widdowson and Spray (*Arch. Dis. Childhood* **26,** *205 (1951)*) presented a detailed analysis of a number of fetuses and stillborn infants. In full term infants fat accounted for about 16 per cent of body weight. Body water, as a fraction of the fat-free body weight, shows a striking decrease during fetal development (from over 90 per cent at the early stages to 82 per cent at full term).

Unavoidably, knowledge gained from direct analyses of the body will increase only slowly. Fortunately, nutritional and chemical research on body composition may utilize several methods applicable to living human beings (*Nutrition Reviews* **9,** *265, 344 (1951)*; **10,** *14, 70, 181, 246 (1952)*).

An important approach is represented by the "isotopic dissection" (F. D. Moore, *Science* **104,** *157 (1946)*). Using radioactive and stable tracer materials injected intravenously and allowed to reach uniform distribution, certain constituents of the body can be measured on the basis of the dilution principle. The volume of the solvent is determined on the basis of the initial concentration and the volume of a solute, which is known, and the concentration of the solute after dilution, which is determined for each individual. The "solvent" may be a fluid (such as the total body water or its extracellular fraction), or it may be a solid constituent of the body. Thus, from the data on the dilution of radioactive potassium in the body's potassium one may calculate the amount of "exchangeable potassium" in the body, a value which is smaller than the "total" potassium (L. Corsa *et al.*, *J. Clin. Invest.* **29,** *1280 (1950)*). The potassium content is of particular interest because it correlates with creatinine excretion and seems to be related to the fat-free body mass.

The general principles involved in the use of tracers (isotopes and color tracers) for the study of body composition, a brief history, and some significant advances made in recent years were reported by I. S. Edelman and his colleagues at the Laboratory for Surgical Research of the Harvard Medical School, directed by F. D. Moore (*Science* **115,** *447 (1952)*). Informative data are provided for normal young adult males and females. Unfortunately, neither the mean ages nor the departure from standard body weight, which might serve as a gross measure of relative obesity, were given. There is no difference in the amount of exchangeable sodium (41.4 and 41.0 mEq per kilogram of body weight for males and females, respectively) and only a small difference in the extracellular fluid measured by thiosulfate dilution (16.8 and 17.5 per cent of body weight). On the other hand, the amount of exchangeable potassium (46.8 versus 40.7 mEq per kilogram of body weight) and of total body water measured by deuterium dilution (62.0 versus 51.5 per cent of body weight), are definitely higher in the males. Using the formula for calculation of body fat from body water (E. N. Rathbun and N. Pace, *J. Biol. Chem.* **158,** *667 (1945)*), one obtains the fat values of 15.3 and 29.6 per cent, indicating a marked sex difference in the fat content of the adult human body. Several case studies of large changes in body weight are presented as an example of the application of these methods for the study of body composition, supplemented by the analysis of changes in lean tissues based on nitrogen balance studies.

In their review of the technics and practical implications of measurement of body water J. D. Hardy and D. L. Drabkin (*J. Am. Med. Assn.* **149,** *1113 (1952)*) noted that the percentage of body water in female subjects appeared lower than for males of corresponding weight and habitus. This is in agreement with the observations of Edelman *et al.* (*loc. cit.*). The average value of body water in 8 healthy men, determined by deuterium dilution, was 60 per cent of their body weight. Using the estimation equation of Pace and Rathbun, this would correspond to 18 per cent of fat. The age of the subjects was not specified. The average value of body water for women was given as 55 per cent of body weight. With reference to the subject's fatness it was noted that the men who, individually, had values in the neighborhood of 60 per cent were well nourished, being neither skinny nor fat. P. Schloerb *et al.* (*J. Clin. Invest.* **29,** *1296 (1950)*), also measuring the total body water by deuterium dilution, reported average values of 61.8 per cent for body water in healthy young adult men and 51.4 in healthy young adult women. The mean ages were 25.4 and 24.7 years, with 19 male and 11 female subjects. The total body values are very close to those reported by Edelman *et al.* (*loc. cit.*). Taking all these data together, there is little doubt left about the "physiological fatness" of the adult women (B. Škerlj, *Arch. f. Frauenkunde* **16,** *235 (1930)*).

A review of the recent literature on water metabolism, with sections on total body water and on the composition of the body, was presented by J. R. Robinson and R. A. McCance (*Ann. Rev. Physiol.* **14,** *115 (1952)*). It is strange that this paper credits authors writing in 1950 with seeing "the possibility of determining fat in the living person without recourse to measurements of specific gravity," while Rathbun and Pace (*loc. cit.*) elaborated the concepts, provided fundamental evidence on the reciprocal relationship between fat and water, and presented an equation for predicting the fat content of the whole animal mass from its water content. Two years later N. Pace, L. Kline, H. K. Schachman, and M. Harfenist (*J. Biol. Chem.* **168,** *459 (1947)*) reported measurements of the total body water in 2 rabbits and one man by the use

of tritium, a radioactive isotope of hydrogen of mass 3.

In a study on the use of tritium for body water determinations, T. C. Prentice *et al.* (*J. Clin. Invest.* **31,** *412 (1952)*) explored the possibilities of this method for routine measurements and extended the scope of investigations to a variety of disease states. Total body water was determined with tritium-labeled water in 57 individuals. Of particular interest are the 4 young (average age 22.2 years) and 15 middle-aged (average age 44.5 years) normal men. They exhibit a marked age difference, with mean body water values of 60.4 and 52.1 per cent, respectively, reflecting the higher fat content of the older men. The calculation of fat is somewhat uncertain due to the fact that the obtained water volume is slightly greater than the true volume, due to the exchange of some of the tritium with labile hydrogen atoms in body proteins and carbohydrates. In the 4 young men the total body water determined by antipyrene dilution was lower than the tritium value (60.4 versus 56.8 per cent). In a group of 10 patients the difference was smaller (56.2 versus 54.6 per cent). The estimation of fat from total body water rests on the assumption that the fat-free body mass has a relatively constant hydration (73 per cent). The authors point out that in pathologic states in which the fat-free body mass cannot be assumed to retain a constant composition, body water determinations are of limited value without an independent method for the measurement of fat.

W. W. Hurst, F. R. Schemm, and W. C. Vogel (*J. Lab. Clin. Med.* **39,** *36 (1952)*) have found that in edematous patients the antipyrene-total-body-water volumes were consistently smaller than the deuterium volumes. The larger the edema, the greater was the difference. This was interpreted as an indication of a difference in the diffusion rates of the two agents throughout the body. In the presence of massive edema, the deuterium method was considered more accurate, but even then care must be taken to allow considerable time until equilibration is reached. Interestingly enough, while the absolute values obtained in 30 patients with edema showed a considerable difference (40.6 liters by antipyrene, 47.7 liters by deuterium, representing 54.6 and 64.2 per cent of body weight), the *changes* in the volume of the edema fluid were characterized satisfactorily by either method (8.6 liters by antipyrene, 9.5 liters by the deuterium method). A part of the weight loss of 12.2 kg. was accounted for by solid tissues.

While antipyrene has many advantages as an agent for the measurement of body water, it also has some limitations. It is bound to plasma proteins (to the extent of about 10 per cent) and in the calculations a correction must be applied for the amount of antipyrene metabolized during the period of measurement. Several consecutive analyses of antipyrene concentration in the plasma are needed. Another substance, abbreviated as NAAP (N-acetyl 4-aminoantipyrene), was found to distribute itself in body tissues in proportion to their water content (B. B. Brodie and J. Axelrod, *J. Pharmacol. Exp. Therap.* **99,** *171 (1950)*). In contrast to antipyrene, less than 3 per cent of this substance is bound to plasma proteins, its metabolism within the body is negligible (0 to 15 mg. of a 1 gram dose over a period of three hours), and it is excreted slowly. The volume in which it is distributed may be calculated from a single plasma and urine sample (Brodie *et al.*, *Proc. Soc. Exp. Biol. Med.* **77,** *794 (1951)*). In 12 normal human subjects in whom the body water measurements were made with both substances, the antipyrene space averaged 1.8 liters higher than the NAAP space. This appears to be a difference in the "wrong" direction, as far as the "true" volume of body water is concerned.

ANTIBIOTICS AS GROWTH STIMULATORS IN RUMINANTS

The observations that dietary supplements of antibiotics would stimulate the growth of animals have received a great deal of attention (*Nutrition Reviews* **8,** *298* (*1950*); **9,** *359* (*1951*); **10,** *211* (*1952*)). Preliminary results, based on excretion rates, have also indicated that antibiotics probably have a sparing effect on the biotin and niacin requirement in man (*Ibid.* **10,** *294* (*1952*)) similar to B-vitamin sparing effects noted in rats (*Ibid.* **10,** *107* (*1952*)).

In a number of these studies it was also emphasized that the type of basal diet employed could have an effect on the ability of antibiotics to stimulate growth. Mice, for example, responded to antibiotics only when the basal diet contained soybean meal or cottonseed meal as the source of protein, whereas no stimulation was noted when the protein of the diet consisted of casein or peanut meal.

L. L. Rusoff, J. A. Alford, and C. E. Hyde (*J. Dairy Sci.* **36,** *45* (*1953*)) have recently investigated the effect of type of dietary protein on the response of young dairy calves to aureomycin. Thirty-six male calves (18 Holsteins and 18 Jerseys) were divided in three groups. These groups were fed respectively a calf starter containing (1) soybean oil meal, (2) cottonseed meal, or (3) degossypolyzed cottonseed meal as the principal protein source. One-half the animals in each group received a 1 per cent supplement of Aurofac A containing 1.8 g. of aureomycin per pound.

All calves received colostrum for the first three days after birth. Whole milk was fed for thirty days at the rate of 1 pound per 10 pounds of body weight. Those receiving Aurofac in the calf starter also had their milk fortified with the antibiotic (50 mg. aureomycin hydrochloride daily per calf). The calf starter feeding was initiated on the eighth day after birth. A medium grade lespedeza hay was also fed from the eighth day. A vitamin A supplement was provided all calves after weaning.

Regardless of the type of protein in the calf starter, the average daily weight gain of calves receiving aureomycin was 10 to 20 per cent greater than the gains of those not receiving the antibiotics. The average daily gain per head was 1.22 pounds for calves receiving antibiotics, and 1.05 for calves receiving the same rations without antibiotic. The growth stimulation was slightly better on the soybean than on the cottonseed meal rations. Removing the gossypol from the cottonseed meal had no effect on growth. Most of this difference in gain appeared after the calves were weaned, indicating that the antibiotic apparently exhibited little or no stimulation when the ration contained milk. There was a slight, although not consistent, improvement in the efficiency of feed utilization attributable to antibiotic supplement. With the soybean meal diet, 2.17 pounds of calf starter were consumed per pound of body weight gain, while the same ration plus antibiotic required 2.04 pounds. The cottonseed meal was less efficiently utilized, requiring about 2.60 pounds to produce a pound of gain in the absence of antibiotic, and 2.30 pounds with antibiotic.

Other workers have reported growth stimulation in calves from antibiotic additions to the ration. In some instances, antibiotics have been observed to prevent scours in young calves, a condition which might slow the growth process. A. M. MacKay, W. H. Riddell, and R. Fitzsimmons (*J. Animal Sci.* **11,** *341* (*1952*)) were able to show, however, that an aureomycin feed supplement produced a significant increase in body weight in calves during the first twelve weeks of life when no scours were noted in either the treated or control animals. The former gained 136 pounds in body weight (average of 10

calves) whereas the latter gained 117 pounds in body weight (average of 5 calves). The ration consisted of a liberal intake of milk, good quality hay, and a calf starter in which soybean oil meal was the principal protein source. These workers found no significant effect of the antibiotic on efficiency of utilization of feed nutrients.

W. R. Murley, N. L. Jacobson, and R. S. Allen (*J. Dairy Sci.* **35,** *846 (1952)*) determined the effect of supplementing a calf ration with crystalline aureomycin hydrochloride (80 mg. per calf daily). The aureomycin-fed calves gained significantly more in weight (25 per cent more) than the control calves during a sixteen-week experimental period. Moreover, these workers found that the treated calves consumed more feed and utilized the digestible nutrients in the feed more efficiently than did control animals. When the aureomycin-fed group was restricted in food intake to the level of intake of those not receiving aureomycin, there was, however, no difference in growth rate between the two groups. Although the antibiotic effected a decrease in the incidence of scours, the improved growth could not be attributed to this factor alone.

After the sixteen-week feeding period the 32 calves were regrouped. Half of those that had previously received the antibiotic were continued on this treatment while the other half were fed the ration without antibiotic. Similarly, half the calves previously on unsupplemented ration were now given the antibiotics while the other half were continued on the unsupplemented ration. During the subsequent twelve-week period, gains in body weight and increases in circumference of chest and of barrel were significantly greater for calves fed antibiotic than for calves receiving no antibiotic. Previous treatment had some effect in that the calves previously fed no antibiotic responded more than did the calves that had received antibiotic during the first sixteen weeks.

J. K. Loosli and H. D. Wallace (*Proc. Soc. Exp. Biol. Med.* **75,** *531 (1950)*) reported that aureomycin in a diet of various milk substitutes, with grain and hay, stimulated the growth of calves from 2 to 8 weeks of age (20 per cent increase in growth). These workers fed the antibiotic at a level of 0.5 g. of aureomycin per 100 pounds of dry matter intake and noted that this treatment reduced the incidence and severity of scours.

In an initial report, J. B. Williams and C. B. Knodt (*J. Animal Sci.* **10,** *144 (1951)*) failed to obtain growth stimulation when the calf starter ration contained a supplement containing aureomycin or streptomycin. Subsequently, it was found that aureomycin supplements of 2, 4, 6, 8, and 10 g. per 100 pounds of feed were beneficial, particularly at the higher levels, in stimulating growth and in controlling scours (S. Bloom and Knodt, *Ibid.* **10,** *1039 (1951)*).

Thus far, no growth-stimulating effects from antibiotic have been reported with sheep. R. W. Colby, F. A. Rau, and J. C. Miller (*J. Animal Sci.* **9,** *652 (1950)*) observed growth depression in lambs when the ration was supplemented with aureomycin, penicillin, and streptomycin (100 mg. per lamb daily, orally). More recently, R. M. Jordan (*Ibid.* **11,** *566 (1952)*) tried aureomycin supplements at levels of 7.2 to 14.4 mg. per lamb daily and noted that the higher level decreased body weight gains. In one trial the antibiotic reduced the incidence of enterotoxemia materially, whereas no effect was noted on this condition in the other three trials.

With regard to the use of antibiotics in the ration of older ruminants, M. C. Bell, C. K. Whitehair, and W. D. Gallup (*J. Animal Sci.* **9,** *647 (1950)*) noted that 600 mg. per head per day of aureomycin hydrochloride caused beef cattle to go "off feed" and develop diarrhea. One-third this level was noted to depress digestibility of feed nutrients.

From the presently published detailed reports concerning the use of antibiotics in the feed of young growing ruminants, the following can be concluded generally: (1) Aureomycin appears to improve growth rate in growing calves when added to calf starter rations that are in conventional use in the dairy industry. Under certain conditions, part of this response appears to be associated with control of scours, a condition which generally is best controlled when good sanitation is practiced. (2) There is some question of the value of antibiotic in improving the efficiency of utilization of feed nutrients, although some results suggest such improvement. (3) Use of antibiotics in lamb rations has not proven of value as growth stimulants. It is clear that much work lies ahead in the establishment of desirable levels of antibiotics and in evaluating their relative usefulness in the nutrition of young ruminants. Most of the work to date with calves has involved the use of aureomycin.

RELATION OF FEEDS TO STABILITY OF TURKEY CARCASS FAT

The ability of carcass fat to resist off-flavor development in storage is of practical importance. This is particularly true in the case of turkeys because seasonal production requires that they be stored longer than other meats. Processing and storage can have rather marked influences on the acceptability and quality of turkey meat, but the type of diet consumed by the turkey may in many instances be of greater importance than processing and storage.

The early studies with hogs (N. R. Ellis and H. S. Isbell, *J. Biol. Chem.* **69,** *219 (1926)*) and with chickens (E. M. Cruickshank, *Biochem. J.* **28,** *965 (1934)*) established the fact that the fatty acid composition of the carcass fats is quite sensitive to changes in dietary fat. Recently, workers at the Western Regional Research Laboratory and the University of California have demonstrated a similar relationship between carcass fat and dietary fat in turkeys (A. A. Klose, E. P. Mecchi, H. L. Hanson, and H. Lineweaver, *J. Am. Oil Chem. Soc.* **28,** *162 (1951)*). Furthermore, these workers noted that the composition of carcass fat had an important influence upon its stability in storage. Two comparisons of 5 per cent dietary levels of coconut and linseed oil indicated that doubling the iodine value of the carcass fat (linseed oil-fed) caused a twenty-five-fold decrease in the stability of the carcass fat.

A more recent study by these workers presents data on the influence of low levels (2 per cent) of dietary fat on carcass composition and on stability of the carcass fat (A. A. Klose *et al.*, *Poultry Sci.* **31,** *354 (1952)*). The experimental diets were, with respect to fat composition, as follows: Diet 1 was a simplified diet containing only 0.5 per cent of fat. Diets 2, 3, 4, 5, and 6 were similar but with the addition of 2 per cent of beef fat, corn oil, linseed oil, sardine oil, and soybean oil, respectively. Diet 7, containing about 5 per cent of fat, consisted of a standard commercial turkey growing ration of mash and grain, the mash containing 5 per cent of fish meal and 0.35 per cent of fish oil. These diets were fed for a seventeen-week experimental period to turkey poults (8 birds per treatment) which had been grown to 2.5 kg. body weight during the previous thirteen weeks on a regular turkey starting mash.

After slaughter, the carcass fats were analyzed spectrophotometrically for equivalent linoleic, linolenic, and arachidonic acid content. Based on these data, iodine numbers, and the estimate that 95.6 per cent of

the fat was fatty acids, the saturated fatty acid and oleic acid contents were calculated.

The stability of the carcass fat was determined by measuring the rate of oxygen uptake in a manometric apparatus in an oxygen atmosphere at 70°C. The results indicated that the linoleic and linolenic acid content of the carcass fat was a function of the levels of these fatty acids in the diet. For example, when the diet fat contained 35 to 42 per cent of linoleic acid, the carcass fat contained 13 to 15 per cent of this acid, whereas diet fat linoleic percentages of 23 or lower resulted in carcass values of 3 to 6 per cent. The oleic acid content of the carcass fat, which in the analysis used also includes hexadecenoic acid, ranged between 46 and 63 per cent of the total fatty acids and was more or less independent of dietary fat composition. This is assumed to be an expression of the ability of the bird to adjust through saturation, desaturation, or synthesis. The percentage saturated fatty acids in the carcass fats ranged between 28 and 30 per cent of the total fatty acids for all groups, despite a wide variation in composition of the diet fats, an indication that the turkey is able to fix this content within narrow levels when diets relatively low in fat are fed.

The carcass fat composition of the group receiving no added dietary fat and the group receiving 2 per cent of beef fat were practically identical, indicating that the fat synthesized by the turkey on a low fat diet is practically identical with that deposited when beef fat is fed. The marked dietary effect on body fat composition of diets containing only 2.5 per cent of fat, as indicated by these studies, would suggest that the composition of turkey fat is mainly dependent upon dietary fat composition, since most commercial turkey feeds contain about 5 per cent of fat.

The fat storage stability tests indicated that storage stability was inversely related to the concentration of unsaturated fatty acids in the depot fats. The contribution toward instability seemed to come mainly from the linolenic acid fraction, except in the case of linseed oil where 5- and 6-double bond fatty acids were considered mainly responsible.

The diet treatments represented three general classes with respect to carcass fat storage stability. The basal low fat diet, the beef fat, and the corn oil diets produced very stable carcasses. The diets containing 2 per cent of soybean oil and the commercial turkey mash plus grain produced moderately stable carcasses, whereas the linseed oil and sardine oil diets produced carcasses of low stability.

The frozen eviscerated carcasses were evaluated initially and after six, twelve, and eighteen months of storage at 0°F. for peroxides and aldehydes in the raw depot fat and for fishy and rancid off-flavors and odors in the roasted carcasses (A. A. Klose *et al.*, *Poultry Sci.* **32,** *82* (*1953*)). Two per cent of sardine meal in the diet produced intense fishiness in the cooked carcass, 2 per cent of dietary linseed oil produced moderate fishiness, while the commercial diet containing about 0.4 per cent of fish oil produced only slight fishy flavors in the cooked carcasses. The results indicated that the tendency of fat to deteriorate in frozen storage may be predicted from fat storage stability tests or the fatty acid composition of the carcass fat.

These studies establish conclusively that the type of dietary fat fed can affect the storage life and eating quality of turkey carcasses. The effects on storage quality, however, were not excessive unless fats were present in the diet that contained appreciable amounts of highly unsaturated fatty acids. Of the dietary fats tested, fish oils would appear to be the most likely source of such fats for producing unstable or off-flavor turkey carcasses under present commercial practices.

NUTRITION AND ALCOHOLISM

The most recent efforts to establish an organic etiology for alcoholism have centered around the theory that deficiencies of certain nutrients may lead to a craving for alcohol. This concept has been most energetically propounded by R. J. Williams, who has included it in the group of diseases which he labels "genetotrophic" (*Nutrition Reviews* **8,** *257 (1950)*). This is defined as a disease which "occurs if a diet fails to provide a sufficient supply of one or more nutrients required at high levels because of the characteristic pattern of the individual concerned." Thus, in his studies of rats, when animals which previously had shown differing degrees of appetite for alcohol were given abundantly supplemented diets, the consumption of alcohol fell markedly in all. From these results the concept has developed that the urge to consume alcohol is conditioned by nutritional deficiency.

This type of study has been reviewed by J. Mardones (*Quart. J. Studies Alcohol* **12,** *563 (1951)*), who has conducted similar investigations. He has compared his work with that of Williams, and of R. A. Brady and W. W. Westerfeld (*Ibid.* **7,** *499 (1947)*), and has concluded that genetic characteristics may indeed lead to increased requirements for certain nutrient factors by certain groups of rats. In the absence of these factors such rats may imbibe alcohol in preference to water. He also emphasizes in his review the role of Factor N_1 which he believes is found in certain types of untreated brewers' yeast, in liver, meat and wheat germ. In his view it is this factor, in association with other vitamins, particularly thiamine, that is most effective in reducing or preventing the increased alcohol intake.

This type of study has recently been more critically repeated by D. Lester and L. A. Greenberg (*Quart. J. Studies Alcohol* **13,** *553 (1952)*), who point out that other investigations have not used an alternative source of calorie-providing materials in addition to alcohol. Accordingly, using basic diets of 46 and 63 per cent sucrose, composed mainly of vitaminized casein and vitaminized Wesson oil, and other similar B vitamin-deficient and B vitamin-free diets containing vitamin-free casein, they have provided albino male rats with access to fat (5.8 per cent homogenized in gum acacia), sucrose solution (11.5 per cent), solid sucrose, and saccharine solution (0.09 per cent, as sweet as sucrose to human taste), in addition to alcohol and water. Groups of 5 or 10 rats were given the 46 per cent sucrose diet with only 7.6 per cent alcohol solution and water as the initial alternatives and then given successively the additional choice of fat, sucrose solution, solid sucrose and saccharine. In one group the succession of fat and sucrose was reversed, the sucrose solution period preceding the fat period. With this diet all extra choice regimens provided a significant lowering of alcohol intake which was particularly striking for the period when sucrose solution was provided. In this period the intake of alcohol expressed as absolute alcohol in milliliters per 100 g. of body weight per day was 0.10 as compared to 0.56 for the control period. Subsequently, the use of a 63 per cent sucrose-vitamin-sufficient diet reduced the intake of alcohol to 0.29 ml. from the 0.56 ml. observed when the diet contained 46 per cent sucrose.

Subsequently vitamin-deficient diets of 46 per cent sucrose were given and alcohol intake was compared with only water as an alternative and with sucrose solution as an added choice. This regimen also yielded a significant reduction in alcohol drinking when sucrose solution was provided. Comparison of the alcohol intake on vitamin-sufficient and vitamin-deficient diets with 63 per cent sucrose content revealed slightly greater intake on the latter diet and this difference was even greater when a vitamin-free diet was used. When this vitamin-free

diet was supplemented with a choice of sucrose solution in addition to the alcohol and water, however, there was a marked drop in alcohol ingestion. Removal of sucrose solution was followed by an increase in alcohol intake even when there was a simultaneous shift from a vitamin-deficient diet to a vitamin-sufficient one.

The uniformity of the decrease in alcohol consumption in these studies when a third choice was added to the usual alcohol and water alternatives is remarkable, particularly in view of the results obtained using saccharine. This suggests that the desire for the supplementary fluid is not necessarily caloric, a suggestion that is supported by the statement of the authors that when sucrose solution was offered, the calories consumed from this source surpassed the calories ingested as alcohol during the control periods.

These results appear to alter significantly the interpretations which can be made of the work of Williams and of Mardones in regard to alcoholism as a genetotrophic disease. It would appear that the disturbance in rats, genetotrophic or otherwise, is the desire for additional calories, or perhaps simply for some palatable fluid in addition to water. The work of Lester and Greenberg does not necessarily invalidate the previously observed relationship between diet and alcohol consumption of rats. Differences in the diets which have been used, such as the sugar content, may be of importance. It does, however, indicate the need for more careful definition of the specificity of the appetite for alcohol under various conditions. The human being can presumably select things other than alcohol if nutritional deficiency does indeed change his appetite. The authors have also pointed to another important difference in rats and human beings. Rats have never been observed to consume sufficient alcohol at one time to become intoxicated. The investigation strongly suggests that there have been deficiencies in the experiments used to demonstrate the genetotrophic concept of alcoholism and emphasizes the difficulties inherent in attempting to transfer the results of animal experiments to the human being.

COMPARATIVE ACTIVITIES OF PYRIDOXAL, PYRIDOXAMINE AND PYRIDOXINE

Most nutrition studies with vitamin B_6 have been conducted with pyridoxine. Pyridoxal and pyridoxamine are the active forms in which this vitamin occurs in natural foodstuffs. The activities of these substances compared with pyridoxine have not been investigated very extensively, although it has been shown that all three forms of the vitamin are equally active for rats and chicks when they are injected or fed separately from the other components of the ration (E. E. Snell and A. N. Rannefeld, *J. Biol. Chem.* **157,** *475 (1945)*). When pyridoxamine or pyridoxal were mixed into rations high in soluble carbohydrates, however, they were found to be distinctly less active for chicks and rats than pyridoxine (P. S. Sarma, Snell, and C. A. Elvehjem, *Ibid.* **165,** *55 (1946)*). The inclusion of aureomycin in such diets resulted in equal activities for all three forms of the vitamin for rats (H. Linkswiler, C. A. Baumann, and Snell, *J. Nutrition* **43,** *565 (1951)*).

The influences of dietary carbohydrates and of aureomycin on the relative activities of the three forms of vitamin B_6 were recently investigated with chicks (P. E. Waibel, W. W. Cravens, and E. E. Snell, *J. Nutrition* **48,** *531 (1952)*). The basal diet used had the following composition in grams per 100 g. of diet: hot alcohol-extracted casein, 18; gelatin, 10; salts, 6; soybean oil, 4; vitamin A and D oil (2,000 I.U. of A and 300 I.U. of D), 0.5; choline chloride, 0.2; inositol, 0.1; L-cystine, 0.3; and carbohydrate, 61. The carbohydrates

used in the diets were glucose, sucrose, autoclaved starch, and a mixture of each of these with lactose (41 parts glucose, sucrose, or autoclaved starch and 20 parts of lactose). The diets were fortified with the following vitamins (milligrams per kilogram of diet): alpha-tocopherol, 3.0; thiamine hydrochloride, 3.0; niacin, 50; calcium pantothenate, 20; biotin, 0.2; menadione, 0.5; folacin, 4.0; para-aminobenzoic acid, 100; riboflavin, 6.0; and vitamin B_{12}, 0.03.

When the diet contained the soluble carbohydrates, glucose or sucrose, pyridoxal and pyridoxamine were less effective than pyridoxine. This confirms the earlier observation. Aureomycin exerted little or no effect on the comparative activities of the three forms of the vitamin when the diet contained glucose or sucrose. In this respect the chick appears to differ from the rat (Linkswiler *et al.*, *loc. cit.*).

When the dietary carbohydrate consisted of autoclaved starch, all three forms of vitamin B_6 were equally active for the chick. This was true in both the presence and the absence of aureomycin.

Aureomycin stimulated growth only when the rations were adequate with respect to vitamin B_6. These results would indicate that the pyridoxine-sparing action of an antibiotic, such as has been reported in rats (*Nutrition Reviews* **10,** *107* (*1952*)), cannot be demonstrated in chicks under the conditions employed by Waibel and associates.

Substantial growth stimulation was noted when 20 per cent of the carbohydrate in the glucose- and sucrose-containing diets was replaced by an equal amount of lactose. The lactose addition, however, did not have any influence on the comparative activities of the three forms of vitamin B_6; pyridoxine still was superior to the others.

The results of Waibel and associates indicate that the three forms of vitamin B_6 are equally active when fed on diets containing autoclaved starch. Under conditions of practical nutrition, the dietary carbohydrate sources are mainly in this form rather than in the form of soluble compounds such as glucose or sucrose. This would suggest that the naturally occurring forms of vitamin B_6 (pyridoxal and pyridoxamine) are equally as active as pyridoxine under practical feeding conditions.

CHEMICAL CHANGES IN CONSTITUENTS OF FLOUR INDUCED BY CHLORINE DIOXIDE

The use of nitrogen trichloride or agene as an improver of flour was found to produce an antimetabolite of methionine, methionine sulfoximine, which elicited toxic manifestations in dogs, rabbits and cats (*Nutrition Reviews* **8,** *14*, *170* (*1950*); H. R. Bentley *et al.*, *Proc. Roy. Soc.* (*London*) *B* **137,** *402* (*1950*)). This finding has made it necessary to utilize other agents for flour improvement. The desired action of these materials is to bleach and artificially "age" the flour, so that it meets the technical requirements of present methods and standards of bread production. The experience with agene warrants caution and careful testing in the adaptation of new agents for this use. One of the agents which has been widely used in Europe is ammonium persulfate. The apparent lack of toxicity of flour treated with this compound has been described (*Nutrition Reviews* **9,** *139* (*1951*)).

A more commonly used flour improver in the United States today is chlorine dioxide. Flour treated with chlorine dioxide has been fed to a variety of species of experimental animals without evidence of acute toxicity or abnormalities of any kind (G. W. Newell *et al.*, *Cereal Chem.* **26,** *160* (*1949*)). It is pointed out by T. Moran, J. Pace, and E. E. McDermott (*Nature* **171,** *103* (*1953*))

that there are limitations in the interpretation of a compound used in food production or manufacture as nontoxic on the basis of animal feeding tests alone. It is their feeling that the biologic tests of toxicity should be supplemented with investigations of the biochemical changes induced in the substance treated, in this instance, flour. They have, therefore, undertaken intensive study of the changes induced in certain of the constituents of flour when treated with chlorine dioxide. This flour improver is applied commercially as a gas in very small concentrations, about 30 parts per million. In the experiments described flour was treated with pure chlorine dioxide or chlorine dioxide containing approximately 30 per cent chlorine. These substances were utilized in concentrations equal to or greater than those applied in flour manufacture.

The lipids of flour were found to interact freely with chlorine dioxide and with chlorine. Oxidation of unsaturated fatty acids occurs as demonstrated by the use of a thiobarbituric acid reagent. Furthermore, it was shown that treatment of an 81 per cent extraction flour with chlorine dioxide in ten times the commercial concentration caused a decrease in the iodine number of the fat. The oxidation value, determined by the thiobarbituric acid test, in untreated flour stored for periods of from three to twelve months was found to be either less or greater than that of flour treated with chlorine dioxide at the commerical level. Apparently the content of natural antioxidants in flour varies and determines the degree of autoxidation of unsaturated fatty acids on standing.

Pure chlorine dioxide applied to flour produces no chlorination of the fat. When free chlorine is present in the gas, chlorination does occur and as much as 40 per cent of the chlorine applied may be recovered in the lipids extracted from the flour.

Evidence was obtained indicating that the oxidation in lipids may be but a step in a sequence of oxidative changes affecting other constituents of flour. When the fat of chlorine dioxide-treated flour was placed on kieselguhr with methionine, there was almost complete oxidation of the methionine to the sulfoxide. This did not occur when the fat from untreated flour and methionine were combined under the same conditions.

The flour utilized most extensively in these studies, 81 per cent extraction, contains wheat germ and accordingly possesses a relatively great content of vitamin E or tocopherols. Commercial treatment of this flour with chlorine dioxide resulted in a loss of 70 per cent of the tocopherol, as determined by chemical tests.

The effect of chlorine dioxide on the proteins of flour was investigated. The conditions imposed upon this type of study are discussed. Although it is of considerable importance to consider the individual proteins of flour, these are obtained only by hydrolysis and the presence of large quantities of carbohydrate makes this difficult, with losses of unknown quantity and production of modified proteins. It was not possible to extract from treated flour a gluten comparable to that derived from untreated flour. The procedure selected, therefore, was to isolate protein fractions from flour by acid hydrolysis and to treat these directly with chlorine dioxide. Amino acids unstable to acid hydrolysis are lost in this procedure. Moreover, only those amino acids which are detected with ninhydrin on the paper chromatogram were investigated.

Fractions present in the hydrolysates of the proteins of treated flour were separated and compared with those similarly derived from untreated flour. Methods of separation included ionophoresis, displacement chromatography on ion-exchange resins, and partition chromatography on kieselguhr. The quantities of chlorine dioxide used in treatment of flour or its protein fractions greatly exceeded those used commercially. This was done in the effort to secure sufficient quantities of modified amino acids to permit

isolation and identification. The protein fractions derived from flour and then treated with the improver were gliadin, zein, lipoprotein complexes, and albumin.

The amino acid residues stable to acid hydrolysis and detectable with ninhydrin which seemed most susceptible to treatment with chlorine dioxide were methionine, cysteine and tyrosine. When pure chlorine dioxide was utilized oxidative reactions occurred with production of cysteic acid and oxides of methionine. The presence of free chlorine permitted formation of halogenated derivatives, particularly mono- and dichlortyrosine. Methionine sulfoximine, the toxic factor produced in agene treatment, was not found in any of the protein fractions treated with chlorine dioxide. The quantitative changes in the essential amino acids produced by chlorine dioxide at commercial levels of application were not great enough to influence the nutrient quality of the diet. It is also unlikely that the oxidative and chlorinated derivatives of the amino acids occurring are present in sufficient quantity to induce toxic effects, unless these compounds are later proven to be extremely potent antimetabolites.

The observations reported are of great interest, in that they add important information to that derived from acute experiments of toxicity in experimental animals. The changes induced and recognized in the constituents of flour by treatment with chlorine dioxide would seem to do no harm to the nutrient quality. The degree of oxidation of unsaturated fatty acids is very little, if any, greater than that occurring in certain untreated flours on storage. The loss in content of tocopherols might be of significance if the flour consumed were demonstrated to contribute importantly in meeting vitamin E requirements of man. The changes in amino acids probably do not reduce significantly the quality of the protein, nor do they give rise to a recognized toxic product. The sum of this information would seem to provide additional support to the claim that chlorine dioxide is a relatively safe improver for use in the manufacture of flour for public consumption.

NUTRITIONAL FACTORS IN THE METABOLISM OF FAT

The importance of atherosclerosis has directed great emphasis toward the investigation of lipid metabolism and the relation of nutrition to abnormalities of metabolism. A group of Russian workers demonstrated the effect of dietary lipids upon vascular disease in rabbits almost 50 years ago (A. I. Ignatovski, *Izvîest Imp. Voyenno-Med. Akad.* (*St. Petersburg*) **26,** *154* (*1908*)). The lesions produced were shown by N. Anitschkow and S. Chalatow (*Zentralbl. f. allg. Path. u. Path. Anat.* **24,** *1* (*1913*)) to be due to the cholesterol content of the eggs fed. Such experimental work, now amply confirmed, coupled with the impressive, if inconstant, relation of hypercholesterolemia states with atherosclerosis in human subjects (D. P. Barr, *Am. J. Med.* **13,** *665* (*1952*); A. B. Gutman, *Ibid.* **14,** *1* (*1953*)) has strongly suggested to both experimentalists and clinicians that the answer to this devastating human disease may lie in a better understanding of lipid metabolism.

G. M. Tomkins and I. L. Chaikoff (*J. Biol. Chem.* **196,** *569* (*1952*)) demonstrated that a restricted caloric intake of the donor animal significantly reduced the capacity of liver slices *in vitro* to synthesize cholesterol. Either carbohydrate, fat, or protein, when given in isocaloric amounts, was capable of restoring the capacity of liver slices to manufacture cholesterol (*Nutrition Reviews* **10,** *345* (*1952*)). Basing their work on the theory that acetate is of importance in the biosynthesis of fat (D. Rittenberg and K.

Bloch, *J. Biol. Chem.* **160,** *417 (1945)*), G. Medes, A. Thomas, and S. Weinhouse (*Ibid.* **197,** *180 (1952)*; *Nutrition Reviews* **10,** *347 (1952)*) used C^{14}-labelled acetate to demonstrate that fasting was associated with a fall in utilization of acetate by liver slices. In addition to this demonstration of the importance of these nutrients, it has been shown that vitamin B_6 deficiency in rats on either high or low fat diets is accompanied by a reduced liver neutral fat content. Vitamin B_6 deficiency was accompanied by a reduction in total body fat, however, only if the deficient animals were fed a low fat diet (*Ibid.* **10,** *21 (1952)*).

Further information relative to lipogenesis in the presence of other B-vitamin deficiencies has recently been offered by K. Guggenheim and R. E. Olson (*J. Nutrition* **48,** *345 (1952)*). Groups of young male rats weighing approximately 40 g. were fed synthetic diets deficient either in pantothenic acid, thiamine, biotin, or choline. Control animals were fed complete diets ad libitum, while a second control group was pair-fed with those on the vitamin-deficient diets. The animals were maintained on these diets until the characteristic signs of each deficiency had developed. At that time a daily dose of 0.1 millimoles per 100 g. body weight of radioactive carboxyl-labelled acetate was administered in the food, for a period of three days. Animals were then killed, and liver, heart, adrenals and serum were examined for the content and radioactivity of fatty acids and cholesterol.

With the exception of those on the choline-deficient diet, the animals all showed a considerable reduction in the rate of growth, when compared not only with their pair-fed controls but also with the control animals on the ad libitum diet.

Thiamine-deficient rats showed decreased total amounts of fatty acids and cholesterol and decreased activity of both fractions in liver, heart and adrenals. Pair-fed controls, however, had relatively comparable changes in these factors. Pantothenic acid deficiency did not affect the fatty acid cholesterol concentrations in liver, serum and heart, although the total C^{14} in the lipids was slightly but not significantly reduced. Adrenals from these deficient animals contained less cholesterol than those of the pair-fed and normal controls, as was expected from the studies of H. W. Deane and J. M. McKibbin (*Endocrinology* **38,** *385 (1946)*). Biotin-deficient animals showed no real difference in fatty acid and cholesterol concentration in liver, heart, serum, or adrenals, nor was there any observed defect in the incorporation of C^{14} into either free fatty acid or cholesterol fractions. Similar findings were obtained in animals deficient in biotin, in that reduction of total cholesterol of fatty acid fractions in the organs or serum could be accounted for by relative inanition.

The choline-deficient rats showed the expected increase in liver fatty acids while both the deficient and pair-fed groups showed a diminished cholesterol content and concentration in heart, serum and adrenals. Liver cholesterol was below normal in the pair-fed group and the deficient group as well. Associated with the augmented amount of liver fatty acid was a comparable increase in radioactivity in this liver fraction. The rate of increase in radioactivity at first exceeded the rate of total liver fat accumulation, suggesting to the authors that inability to metabolize synthesized fat may become increasingly severe as choline deficiency progresses. The high radioactivity in liver fatty acids of choline-deficient rats was a result of the high concentration of total liver fat and was not interpreted as a specific alteration in ability to utilize acetate.

The authors comment on the puzzling lack of a specific effect of pantothenic acid deficiency. It was anticipated that a sufficient lack of this vitamin in the diet would reduce coenzyme A activity and thereby interfere with certain acetylation

processes in fatty acid synthesis. The diet may not have reduced the tissue level of pantothenate and of coenzyme A below a hypothetic minimum necessary to induce such a metabolic defect.

These data failed to demonstrate a specific metabolic defect in the synthesis of fat or cholesterol from labelled acetate in deficiency of pantothenic acid, biotin, choline, or thiamine. It is of interest to note that in regard to the latter vitamin, these data are in close accord with the findings of Medes *et al.* (*loc. cit.*), who concluded that thiamine had no influence on tissue utilization of labelled acetate in the synthesis of fatty acids. The findings demonstrate and confirm the previous observations, that inanition will greatly hinder fat synthesis.

THE SALT HORMONE OF THE ADRENAL CORTEX

One of the most constant manifestations of adrenal cortical insufficiency is an inability of the kidney to retain sodium and excrete potassium. This defect leads to sodium depletion, decreased blood volume, hypotension and possible death in many animal species unless the diet is supplemented with sodium salts. The isolation and identification of desoxycorticosterone from beef adrenal cortices by T. Reichstein and J. von Euw (*Helvet. chim. acta* **21,** *1197* (*1938*)) was soon followed by the demonstration of its physiologic properties. Many investigators using a variety of assay systems have shown that desoxycorticosterone acts primarily on electrolyte metabolism with minimal action on carbohydrate and protein metabolism. For a while it was assumed that desoxycorticosterone itself was the active hormone in the body which regulated electrolyte metabolism.

Fractionation of adrenal cortical extracts by solvent partition and fractional crystallization technics early showed that much of the activity of adrenal cortical extracts with respect to salt metabolism resided in an amorphous fraction with physical properties strikingly different from desoxycorticosterone (M. H. Kuizenga, "The Chemistry and Physiology of Hormones," *p. 57, American Association for the Advancement of Science, Washington, D. C.* (*1944*)).

Desoxycorticosterone itself appears to be present in extremely small amounts in adrenal cortical extracts and its significance has been questioned. Reichstein's finding of desoxycorticosterone in beef adrenals has been confirmed using the technics of paper chromatography of A. Zaffaroni and R. B. Burton (*J. Biol. Chem.* **193,** *749* (*1951*)). A rough quantitative estimate indicated that this substance is present to the extent of about 10 to 25 micrograms per pound of adrenal glands. This concentration is approximately one fortieth of the amount of corticosterone found in the same extracts. All but two of the chromatographic spots found on the paper chromatogram could be assigned to previously identified adrenal cortical steroids.

The presence of a new highly active mineralocorticoid has been claimed by H. M. Grundy, S. A. Simpson, J. F. Tait, and M. Woodford (*Acta Endocrinologica* **11,** *199* (*1952*)). This group of workers has fractionated beef adrenal cortical extract (Eucortone) by the paper chromatographic technics of Zaffaroni and Burton. Steroids from limited areas of paper chromatograms were eluted and assayed for their salt-retaining potency by the method of Simpson and Tait (*Endocrinology* **50,** *150* (*1952*)). In this method, immature adrenalectomized rats are injected with Na^{24} and K^{42}. Urine is collected for two hours after the injection of the test substance. By suitable screening it is possible to measure independently the two radioactive elements. The ratio of Na^{24}/K^{42} in the urine is a sensitive index of desoxycorticosterone-

like substances. Nearly all the mineralocorticoid activity of adrenal cortical extract was found in a limited area of the paper chromatogram. The mobility of the mineralocorticoid resembled cortisone (Kendall's Compound E), but complete separation by paper chromatography was achieved with small quantities of adrenal extract. Comparison of the unknown region with the mobilities of known active adrenal steroids failed to identify the mineralocorticoid.

Potent mineral activity was found in the same area of paper chromatograms of hog adrenal cortical extract. Preliminary observations are also reported on blood which had perfused monkey adrenal glands for forty-five minutes. The authors estimate that the equivalent of 25 micrograms of desoxycorticosterone acetate (DOCA) had been secreted. It is also stated that a similar mineralocorticoid was obtained from blood which had perfused dog adrenals.

Some physical and chemical properties of the mineralocorticoid are recorded. It proved to be sensitive to heat, weak acids and alkalis. It was also inactivated by ultraviolet light and acetylation. These properties resemble those of the amorphous fraction of O. Wintersteiner and J. J. Pfiffner (*J. Biol. Chem.* **116,** *291 (1936)*). A rough estimate of the amount of steroid present in the active region of the paper chromatogram suggests that the mineralocorticoid is twenty-four times more active than DOCA.

If the studies of Grundy and co-workers can be confirmed, they represent a substantial advance in the identification of the cortical hormones active in mineral metabolism. With this beginning, the eventual isolation and identification of the adrenal mineralocorticoid should be possible.

PROTEIN SYNTHESIS BY THE PITUITARY

The uptake of labeled amino acids by the proteins of either live animals or tissue preparations is becoming a widely used tool of investigation. Accumulated evidence shows that the labeling of proteins can be almost universally attributed to actual synthesis of peptide bonds. For example, it has been shown that the label cannot be removed by dialysis. Similarly, removal by the classic reagent, ninhydrin, of radioactive carbon introduced in the carboxyl group is only possible if the acid group of the labeled amino acid is first liberated by hydrolysis.

A number of types of labels have been used in such experiments. Radioactive sulfur, introduced as part of the indispensable amino acid methionine, has been found to be especially convenient. It has been observed in particular (*Nutrition Reviews* **11,** *15 (1953)*) that when radioactive methionine is administered to dogs it is rapidly incorporated into tissue proteins. The rate at which the labeled amino acid is integrated into the molecules depends on the organ considered. The pattern of incorporation of S^{35} in order of decreasing activity is as follows: intestinal mucosa, pancreas, spleen, kidney, plasma, liver, testes, heart, brain and muscle.

A recent report (J. B. Melchior and M. N. Halikis, *J. Biol. Chem.* **199,** *773 (1952)*), describing the uptake of S^{35}-labeled methionine by tissues rather than by whole animals, adds to our information by including the pituitary, studied under various physiologic conditions, in the list of organs in which rates of protein synthesis have been measured by this technic.

The Chicago workers used albino rats as their experimental animals. The subjects were killed by a single blow at the back of the neck, the pituitaries were removed, weighed, split in two and incubated with

radioactive methionine. After the reaction had been stopped, contents of the incubation flask were homogenized and the proteins precipitated, washed and hydrolyzed. Sulfur-containing amino acids other than methionine were carefully removed and the radioactivity of the remaining methionine was determined. Liver slices were used as an object of comparison to obtain relative rates of protein synthesis.

Pituitary tissue was found to be much more active than liver tissue in incorporating methionine. On a dry weight basis, the ratio was of the order of 6:1, which would make hypophyseal tissue the most active yet described. The anterior lobe appeared to be somewhat more active than the posterior.

Age is a factor in influencing this rate of synthesis, with the glands of very young animals about one-third more active than those of old adults, a variation which the authors surmise is related to a relative decrease in the production of growth hormone as the animals become older. The activity of the male and female gland is essentially the same per given weight of tissue, but female pituitaries are about 30 per cent larger than male glands. The various stages of the estrous cycle were not observed to have any effect on synthetic rates. Pregnancy exerted little influence, but a marked drop in incorporation of labeled methionine was observed in lactation.

The authors recognized that, while possible explanations may be suggested for the observed phenomena, a detailed interpretation of the variations of synthetic capacity in different physiologic states must follow determination of which proteins become labeled. Progress on this important aspect of the metabolism of such a central regulatory center will be of great interest.

VITAMIN B_{12}, FOLACIN AND METHYL GROUP SYNTHESIS

The demonstration that methyl groups can be synthesized to some extent in the animal body was made by V. du Vigneaud, S. Simmonds, J. P. Chandler, and M. Cohn (*J. Biol. Chem.* **159,** *755 (1945)*). The Cornell workers showed that deuterium was incorporated into the methyl groups of the choline of body tissues when heavy water was administered to the experimental animals. Confirmation and extension of these results were given by du Vigneaud and his collaborators, as well as by workers in a number of other laboratories (*Nutrition Reviews* **10,** *27 (1952)*).

In more recent work, following the observations of W. Sakami and A. D. Welch (*J. Biol. Chem.* **187,** *379 (1950)*) and of J. A. Stekol, K. W. Weiss, and S. Weiss (*Fed. Proc.* **10,** *252 (1951)*), it was shown by W. G. Verly, J. M. Kinney, and V. du Vigneaud (*J. Biol. Chem.* **196,** *19 (1952)*; *Nutrition Reviews* **10,** *285 (1952)*) that folacin stimulated the synthesis of the methyl group in choline from methanol. They also showed that leucovorin, a factor known to stimulate the growth of *Leuconostoc citrovorum* (*Nutrition Reviews* **9,** *24 (1951)*), acted in a manner similar to folacin.

Meanwhile, evidence accumulated that vitamin B_{12} also intervened in the synthesis of methyl groups. Early indications showing the role of vitamin B_{12} in the metabolism of methionine have been reviewed previously (*Nutrition Reviews* **8,** *301 (1950)*). More recently, it was shown that vitamin B_{12} was also involved in the synthesis of creatine (P. Fatterparker, U. Marfatia, and A. Sreenivasan, *Nature* **167,** *1067 (1951)*) and of N^1-methylnicotinamide, the methylated excretion derivative of nicotinamide (I. E. Liener and M. O. Schultze, *J. Nutrition* **46,** *223 (1952)*).

In order to determine the comparative effect of folacin, synthetic citrovorum factor,

and vitamin B_{12} on the biosynthesis of methyl groups, L. S. Dietrich, W. J. Monson, and C. A. Elvehjem (*J. Biol. Chem.* **199,** *765 (1952)*) studied the influence of these vitamins on the ability of deficient rats to methylate nicotinamide.

All their animals were fed purified diets. The folacin regimen included sulfasuxidine; the vitamin B_{12}-deficient diet was based on corn and soybean meal and included a small supplement of iodinated casein. Dietrich and co-workers found that the folacin-deficient rats lacked the ability to methylate efficiently and to excrete as N^1-methylnicotinamide the large doses of nicotinamide (100 to 300 mg. per kilogram of body weight) which were administered every twenty-four hours during the experimental period. A single injection of 10 micrograms of folacin produced an immediate and significant increase in the urinary output of methylnicotinamide of folacin-deficient rats receiving 140 mg. per kilogram of nicotinamide daily. No effect was noted with doses of 10 or 100 micrograms of synthetic leucovorin administered under the same conditions.

Vitamin B_{12}-deficient rats, also receiving 140 mg. of nicotinamide per kilogram of body weight, excreted significantly lower levels of methylnicotinamide as compared to their controls. Under similar conditions, the administration of 4 micrograms of vitamin B_{12} produced no significant increase in the level of methylnicotinamide excreted until approximately one week after the initial supplementation.

As concerns the lack of effect of synthetic leucovorin, Dietrich and his collaborators speculate on the identity of this product with the naturally occurring *Leuconostoc citrovorum* factor. From the difference in lag duration between the action of folacin and that of vitamin B_{12}, Dietrich and co-workers conclude that, while folacin may be the immediate precursor to the physiologic activator or coenzyme involved in the methylation of nicotinamide or in the synthesis of methyl groups, vitamin B_{12} must play a more indirect and complex role.

It is of interest to note that a similar conclusion was derived, in a previous review (*Nutrition Reviews* **10,** *104 (1952)*), from consideration of studies of the growth and hematologic responses of vitamin B_{12}-deficient pigs to the administration of folacin and of methionine. It was then suggested that vitamin B_{12} might well be concerned, among other roles, with the metabolism of folacin and through this intermediary with a number of physiologic functions, hematopoiesis in particular.

That there is a close relation between the action of the two vitamins on methyl group synthesis and on metabolism generally seems demonstrated by an increasing number of experimental and clinical observations. Elucidation of the mechanism of this interrelationship must await further work.

THE INFLUENCE OF RAPESEED OIL ON ADRENAL CHOLESTEROL

T. H. Kennedy and H. D. Purves (*Brit. J. Exp. Path.* **22,** *241 (1941)*) made the fundamental observations that goiter could be produced in rats by the administration of a diet containing rape seeds. This finding greatly stimulated research in goitrogenic agents and resulted in the discovery of suitable antithyroid drugs. Incident to the original studies it was observed that the adrenal cortices of rats on a rapeseed diet became large and pale due to an increased deposition of lipid.

This observation has been confirmed by K. K. Carroll (*Endocrinology* **48,** *101 (1951)*), who has fractionated rapeseed to identify the factor in rapeseed responsible for the adrenal cortical change. Activity was found to reside in the oil and was definitely independent of the antithyroid factor believed to be L-5-vinyl-2-thiooxazolidone. The in-

corporation of 25 per cent of rapeseed oil in the diet of rats resulted in a three- or four-fold increase in adrenal cholesterol after four weeks. A number of other oils were assayed for their ability to increase adrenal cholesterol. Turnip seed oil and cod liver oil showed a lesser activity than rapeseed oil in increasing adrenal cholesterol, and other oils tested were only slightly active. The cholesterol content of the liver and ovaries increased on a rapeseed diet, but not that of the other tissues tested. Following hypophysectomy the expected increase in adrenal cholesterol could no longer be produced by rapeseed.

Recently the component of rapeseed oil which is responsible for the increased cholesterol content of the adrenals has been identified by K. K. Carroll (*J. Biol. Chem.* **200,** *287 (1953)*). He showed that the activity could be destroyed by hydrogenation. After saponification and formation of the methyl ester the active component could be concentrated by fractional distillation into a fraction with a boiling point at 175 to 194°C. No activity resided in the non-saponifiable fractions. Of the known fatty acids of rapeseed oil the properties determined fitted either eicosenoic or erucic acid (monounsaturated fatty acids containing 20 and 22 carbon atoms respectively). Purified samples of the methyl esters of these two fatty acids were prepared and fed to rats. Erucic acid, methyl ester, proved to be active in the test system, but eicosenoic acid, methyl ester, in concentrations as high as 20 per cent of the diet, was without effect. Nervonic acid, which is the next member of the series of homologous monounsaturated fatty acids, was found to be less active than erucic acid. The erucic acid of rapeseed oil is in the cis configuration. Trial of the trans form of erucic acid, brassidic acid, showed that this compound was probably inactive.

The physiologic effects of rapeseed oil administration on endocrine function of the rat have been studied by K. K. Carroll and R. L. Noble (*Endocrinology* **51,** *477 (1952)*). Young male rats were fed a diet containing 25 per cent rapeseed oil for four to eight weeks and were subsequently subjected to a variety of stresses requiring increased adrenal cortical function. The performance of the rats on the experimental diet was compared to that of rats ingesting the same diet without rapeseed oil. No significant difference was noted between the rats on the special diet and those on the control diet with respect to response of the blood sugar to fasting, insulin sensitivity and ability to sustain water diuresis after water loading. Male rats fed 25 per cent rapeseed oil diet, however, failed to survive exposure to cold. The authors were unwilling to attribute this failure to adrenal insufficiency because cortisone acetate, desoxycorticosterone acetate, adrenocorticotropic hormone, or saline extracts of anterior pituitaries failed to protect the rapeseed oil-fed rats. It was noted that the rats on the experimental diet did not appear so vigorous as the controls, and the sensitivity to cold appeared to be a non-specific loss of resistance. Following the removal of one adrenal there was a lowering of the content of cholesterol and ascorbic acid in the remaining adrenal which was the same in both the treated and control groups.

In further observations the rapeseed oil-treated rats were subjected to stimuli which are known to affect adrenal size and composition. The responses to ACTH, desiccated thyroid feeding, high protein diet, the growth of metastatic tumors, stilbestrol and cortisone administration were essentially similar for the treated and control groups.

In summary, it seems well established that the increase in adrenal size and cholesterol which follows rapeseed feeding is not attributable to the goitrogenic factor, but to erucic acid which is also a constituent of rapeseed oil. The marked alteration which occurs in adrenal cortical composition does not seem to be associated with any alteration in adrenal function and such adrenals respond normally to stimulation. The mechanism whereby erucic acid brings about adrenal cholesterol deposition remains to be discovered.

NOTES

Letter to the Editor

Dear Sir:

Your critical review entitled "Association of Liver Damage with Obesity" which appeared in the November issue of *Nutrition Reviews* (**10,** *325 (1952)*) indicts my use of body weight as a guide to bromsulfalein dosage in the use of that dye as a clearance test of hepatic function, and suggests that surface area would have been a more accurate guide in obese individuals. Your review does allow that a majority of the obese subjects would have shown liver damage in any event, but questions the significance of the retention observed in the remainder.

I should like to call your attention to the fact that the two references cited as a basis for the preferred use of surface area over weight in this connection fail to provide any evidence for such choice, offering merely an a priori assumption. Whether blood volume correlates best with surface area or weight was investigated, however, by J. G. Gibson and W. A. Evans (*J. Clin. Invest.* **16,** *317 (1937)*), who found neither to be accurate in obese persons, the blood volumes in such persons being higher than average if referred to surface area, and lower than average if referred to body weight. In any case, wide individual differences exist in normal blood volumes, with an extreme range of about 30 per cent above and below average values.

Furthermore, all of this discussion is predicated on the assumption that blood volume is the significant factor, because of dilution of the dye, in measurement of clearance as a function of the liver. It is more likely true that dilution of the dye is of relatively little importance since the normally functioning liver should completely clear the blood plasma of dye within the allotted time of the test. The limiting factor is therefore not dilution, but liver function.

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Excretion of Amino Acids in Liver Disease

Several studies have shown abnormalities in the urinary excretion of amino acids by patients with various forms of liver disease (*Nutrition Reviews* **7,** *71 (1949)*), especially in those with Wilson's disease (*Ibid.* **7,** *115 (1949)*; **8,** *252 (1950)*).

Further studies have been carried out by G. J. Gabuzda, R. D. Eckhardt, and C. S. Davidson (*J. Clin. Invest.* **31,** *1015 (1952)*). Twenty patients with advanced cirrhosis were studied. Although there were some abnormalities in the patients with liver disease, the differences in quantity and pattern, compared with normal individuals, were not great. The most marked deviations from normal were increased excretions of methionine and tryptophan, and a decreased loss of isoleucine in the urine of the patients. A high protein intake did not increase greatly the urinary amino acid excretion. As improvement in liver disease occurred, there was a gradual, progressive, although again not great, decrease in the urinary excretion of the amino acids studied.

Effect of Hypophysectomy on Cholesterol Synthesis

The work of G. M. Tomkins and I. L. Chaikoff (*J. Biol. Chem.* **196,** *569 (1952)*), showing that the state of nutrition influenced the rate of incorporation of acetate into cholesterol by liver slices, has been discussed previously (*Nutrition Reviews* **10,** *345 (1952)*). In particular, these workers showed that fasting considerably reduced the rate

of cholesterol synthesis. Equivalent amounts of carbohydrates, fats and proteins stimulated synthesis to a similar degree. Because the steroid-producing endocrines (ovary, testes and adrenal) are controlled by hypophyseal secretions, Tomkins and Chaikoff speculated that the pituitary gland might also influence synthesis of cholesterol in the liver. To test this hypothesis, Tomkins, Chaikoff, and L. L. Bennett (*J. Biol. Chem.* **199,** *543 (1952)*) compared the ability of liver slices from hypophysectomized rats and from sham-operated controls to incorporate radioacetate into cholesterol.

In a first experiment both hypophysectomized and control groups were fed *ad libitum*; the hypophysectomized group showed reduced cholesterogenesis, but food intake and intestinal absorption rates also were depressed, making it difficult to draw definite conclusions. In a second series, both types of animals were fasted for twenty hours before being killed. The fact that cholesterogenesis was again relatively decreased in hypophysectomized animals was taken to indicate that lack of a hormone was also an important factor in the impairment in cholesterol synthesis.

Recent Nutrition Books

Nutrition and Diet in Health and Disease. James S. McLester and William J. Darby. 6th edition. W. B. Saunders Company, Philadelphia and London, 1952. Pp. 709.

Man's Foods. L. B. Jensen. The Garrard Press, Champaign, Illinois, 1953. Pp. 278. Price: $4.50.

Nutrition in India. V. N. Patwardhan. Published by J. C. Patel for The Indian Journal of Medical Sciences. Sole Distributors: Hind Kitabs Limited, 261–263 Hornby Road, Bombay. Pp. 345.

Lind's Treatise on Scurvy. A Bicentenary Volume containing a reprint of the First Edition of "A Treatise of the Scurvy" by James Lind, M.D., with Additional Notes. Edited by C. P. Stewart and Douglas Guthrie. Edinburgh University Press. Sole Agent: Oliver and Boyd Ltd., Tweeddale Court, Edinburgh; 98 Great Russell Street, London, W. C. 1.

Industrial Wastes. Their Disposal and Treatment. Willem Rudolfs, Editor. Reinhold Publishing Corporation, 330 West Forty-Second Street, New York 36, New York, 1953. Pp. 525. Price: $12.00.

Recent Advances in Nutrition Research. With Emphasis on the Newer B Vitamins. Proceedings of the Scientific Sessions of the Seventh Annual Meeting, The National Vitamin Foundation, Inc. A. E. Axelrod, B. F. Chow, T. J. Cunha, R. L. Davis, R. W. Heinle, W. A. Krehl, R. W. Luecke, E. P. Ralli, W. Schreiner, R. B. Schultz, R. W. Vilter, H. D. Wallace, D. R. Weir, and R. W. Winters. The National Vitamin Foundation, Inc., New York, 1952. Price: $1.50.

A Trail of Research. Vincent du Vigneaud. Cornell University Press, Ithaca, 1952. Pp. 191. Price: $3.25.

Therapeutic Meal Plans: A New Diet Manual. Compiled by members of the Department of Nutrition and Dietetics at the University of Kansas School of Medicine and edited by Ruth Gordon and others. University of Kansas Press, Lawrence, Kansas, 1952. Price: $3.00.

A Survey of the Literature of Dental Caries. Prepared for the Food and Nutrition Board, National Research Council, under the supervision of the Committee on Dental Health, by Guttorm Toverud, Sidney B. Finn, Gerald J. Cox, Charles F. Bodecker, and James H. Shaw. Publication 225, National Research Council, Washington, D. C., 1952. Price: $3.00. Order from the Publications Office, National Research Council, 2101 Constitution Avenue, Washington 25, D. C., with remittance payable to the National Academy of Sciences.